L7 0 L4 AND MPB64

=> s MPB44 or mpb45 or mpb51 or mpb59 or mpb70 or mpb80 or mpb84

L8 255 MPB44 OR MPB45 OR MPB51 OR MPB59 OR MPB70 OR MPB80 OR MPB84

=> s l4 and l8

L9 4 L4 AND L8

=> d l9 ab 1-4

L9 ANSWER 1 OF 4 BIOSIS COPYRIGHT 2000 BIOSIS
AB **MPB70** and MPB83 are homologous cross-reactive secreted mycobacterial proteins with very limited species distribution. The expression of these two proteins was compared between several substrains of Mycobacterium bovis BCG, virulent M. bovis and Mycobacterium **tuberculosis** H37Rv. A polyclonal antibody specific for **MPB70** in Western blotting, and a monoclonal antibody, MBS43, found to be specific for MPB83 in ELISA and Western blotting, were used for the comparison. The previously established pattern of high- and low-producing substrains of BCG for **MPB70** is only partially applicable for MPB83. **MPB70** low-producing strains are also MPB83 low-producing, but the expression of MPB83 is much more variable than the expression of **MPB70** in the **MPB70** high-producing strains. Purified MPB83 (23 kDa) was found to be glycosylated. A band in SDS-PAGE at 1-2 kDa lower than that of purified MPB83 may represent unglycosylated MPB83. Furthermore, it was confirmed that purified **MPB70** (22kDa) is unglycosylated. There is cross-reactive antigen at 26kDa. The MPB83 related antigen at 26 kDa was found to be the most abundant. These findings indicate greater heterogeneity between different substrains of BCG than previously realized. Virulent M. bovis produce and secrete large amounts of **MPB70** and MPB83 while both these proteins occur in a far lower concentration in M. **tuberculosis**.

L9 ANSWER 2 OF 4 BIOSIS COPYRIGHT 2000 BIOSIS
AB The presence of mycobacteria in blood culture fluids (BACTEC) of AIDS patients with positive growth indices (GIs, gt 20 U) was investigated by using a multiplex PCR to detect and identify members of the genus Mycobacterium, M. avium, M. intracellulare, and M. **tuberculosis**. Three different methods of extracting mycobacterial DNA from blood culture fluid were compared for use with the multiplex PCR. Mycobacterial cells were pelleted from a small aliquot of blood culture fluid by centrifugation, and the DNA was extracted from cells by heat lysis or a sodium iodide-isopropanol or a phenol-chloroform method. DNAs of different sizes were amplified from a region of the **MPB70** gene of M. **tuberculosis** (372 hp) and from a region of the 16S rRNA gene of members of the genus Mycobacterium (1,030 bp), M. intracellulare (850 bp), or M. avium (180 hp) as a multiplex PCR in a single tube. The amplified DNA products were detected by agarose gel electrophoresis and ethidium bromide staining in all 41 (100%) positive cultures after sodium iodide-isopropanol extraction, in 18 (44%) after heat lysis, and in 5 (12%) after phenol-chloroform extraction. Of the 41 positive cultures, 38 were identified as M. avium and 2 were identified as M. intracellulare by

both routine methods and multiplex PCR. The remaining mycobacterium was identified as M. intracellulare by routine methods and as M. avium by the multiplex PCR. Another six blood cultures that were negative for the presence of acid-fast bacilli after Ziehl-Neelson staining were also negative by PCR. The study shows that the multiplex PCR is a useful method for the detection and identification of either M. avium or M. intracellulare in small samples of cultured BACTEC 13A fluid with positive GIs ranging from 21 to 999 U. The average time to a positive GI was 18 days (median, 13 days) and ranged between 8 and 42 days. The multiplex PCR may permit cultured mycobacteria to be identified at an earlier stage than the routine methods which have been adapted for use with the BACTEC system. The results also show that the method selected for extracting mycobacterial DNA from blood culture fluids is crucial for providing sensitive and accurate PCR results.

L9 ANSWER 3 OF 4 BIOSIS COPYRIGHT 2000 BIOSIS

AB Among the first proteins encountered by the host immune system upon infection or vaccination with mycobacteria are those secreted by the bacillus during growth. The antigen 85 complex of Mycobacterium bovis bacillus Calmette-Guerin (BCG) is composed of three closely related members. The mature 85B protein of M. bovis (**MPB59**) has a high degree of amino acid identity with the M. bovis 85A protein (76%) and the Mycobacterium **tuberculosis** 85B (99%) and 85A (76%) proteins. We have examined the regions of **MPB59** which stimulate human T- and B-cell responses by use of a set of 28 synthetic peptides, 20 amino acids (aa) in length and overlapping by 10 aa. Initial proliferative assays with recombinant **MPB59** demonstrated that peripheral blood mononuclear cells from 95% of BCG vaccinees and 52% of **tuberculosis** patients responded to the whole mature protein. Peripheral blood mononuclear cells from **MPB59** responders, but not nonresponders, were stimulated by peptides in a dose-dependent fashion. Five peptides were reactive in more than half of the **MPB59** responders. The T-cell-reactive regions were essentially identical in the M. bovis and M. **tuberculosis** 85B proteins. Subjects with a variety of HLA-DR phenotypes responded to a number of these peptides. The dominant T-cell-reactive regions were distinct from the peptides recognized by sera from **tuberculosis** patients (aa 71 to 100) and the murine monoclonal antibody HYT27 (aa 61 to 90). The region reactive with antibodies overlapped part of the **MPB59** sequence recently shown to participate in the binding of **MPB59** to fibronectin.

L9 ANSWER 4 OF 4 BIOSIS COPYRIGHT 2000 BIOSIS

AB Bovine **tuberculosis** remains a serious problem in several regions, partly due to a lack of specific diagnostic tests. The aim of this study was to identify bovine T-cell epitopes for defined Mycobacterium bovis antigens using an experimental model of the natural disease. Panels of synthetic peptides (16-mers with five residue overlaps) were produced from published amino acid sequences for **MPB70**, the 19,000 MW antigen and MPB57. In vitro. In lymphocyte proliferation assays were used to identify T-cell epitopes. Lymphocytes from experimentally infected cattle proliferated in response to five epitopes (residues 88-105 and 144-163 for **MPB70**; 1-16 and 67-84 for the 19,000 MW antigen; and 85-100 for MBP57). These epitopes were not recognized by control, non-infected animals, but were recognized by field reactors to intradermal tuberculin testing. All five epitopes were recognized by three different breeds of cattle (Friesian, Charolais and Simmental). In addition, the bovine T-cell epitopes identified for the 19,000 MW antigen in this study

were similar to epitopes previously reported for man and mouse. Thus, as well as identifying candidate reagents for improved diagnostic tests and vaccination, this study provides evidence for genetic promiscuity in T-cell recognition of major mycobacterial epitopes.

=> d his

(FILE 'HOME' ENTERED AT 15:52:17 ON 14 JUN 2000)

FILE 'MEDLINE, SCISEARCH, BIOSIS, EMBASE' ENTERED AT 15:52:51 ON 14 JUN 2000

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L1            0 S MPB64 AND (TWEEN 80)
L2            0 S (TRANSDERMAL DEVICE?) AND TUBERCULOSIS AND ANTIGEN?
L3            0 S (TRANSDERMAL DEVICE?) AND TUBERCULOSIS
L4         3410 S (TRANSDERM? OR TRANSPORT?) AND TUBERCULOSIS
L5            0 S L4 AND (TOPIC? APPLICATION)
L6            9 S L4 AND (TOPIC?)
L7            0 S L4 AND MPB64
L8          255 S MPB44 OR MPB45 OR MPB51 OR MPB59 OR MPB70 OR MPB80 OR MPB84
L9            4 S L4 AND L8
```

=> s l8 and transderm?

L10 0 L8 AND TRANSDERM?

=> s l8 and skin

L11 43 L8 AND SKIN

=> d l11 ti 1-43

L11 ANSWER 1 OF 43 MEDLINE
TI Cell mediated and humoral immune responses of white-tailed deer experimentally infected with Mycobacterium bovis.

L11 ANSWER 2 OF 43 MEDLINE
TI [Examination of protein MPB 70 properties in BCG substrains used for the production of Polish antituberculosis vaccines].
Badanie obecnosci bialka MPB 70 w podszczepie BCG uzywanym do produkcji Polskiej szczepionki przeciwgruzliczej.

L11 ANSWER 3 OF 43 MEDLINE
TI T-cell recognition of mycobacterial proteins **MPB70** and MPB64 in cattle immunized with antigen and infected with Mycobacterium bovis.

L11 ANSWER 4 OF 43 MEDLINE
TI The immunological response of llamas (Lama glama) following experimental infection with Mycobacterium bovis.

L11 ANSWER 5 OF 43 MEDLINE
TI Sequence heterogeneity of an **mpb70** gene analogue in Mycobacterium kansasii.

L11 ANSWER 6 OF 43 MEDLINE
TI Differential T cell responses to mycobacteria-secreted proteins distinguish vaccination with bacille Calmette-Guerin from infection with Mycobacterium tuberculosis.

L11 ANSWER 7 OF 43 MEDLINE
TI Identification of a novel 27-kDa protein from Mycobacterium tuberculosis culture fluid by a monoclonal antibody specific for the Mycobacterium tuberculosis complex.

L11 ANSWER 8 OF 43 MEDLINE
TI Purification of **MPB70** and production of specific monoclonal antibodies.

L11 ANSWER 9 OF 43 MEDLINE
TI Protein G-based enzyme-linked immunosorbent assay for anti-**MPB70** antibodies in bovine tuberculosis.

L11 ANSWER 10 OF 43 MEDLINE
TI Properties of proteins MPB64, **MPB70**, and **MPB80** of Mycobacterium bovis BCG.

L11 ANSWER 11 OF 43 MEDLINE
TI Comparative studies with various substrains of Mycobacterium bovis BCG on the production of an antigenic protein, **MPB70**.

L11 ANSWER 12 OF 43 MEDLINE
TI Specific **skin**-reactive protein from culture filtrate of Mycobacterium bovis BCG.

L11 ANSWER 13 OF 43 SCISEARCH COPYRIGHT 2000 ISI (R)
TI Cell mediated and humoral immune responses of white-tailed deer experimentally infected with Mycobacterium bovis

L11 ANSWER 14 OF 43 SCISEARCH COPYRIGHT 2000 ISI (R)
TI Development of diagnostic reagents to differentiate between Mycobacterium bovis BCG vaccination and M-bovis infection in cattle

L11 ANSWER 15 OF 43 SCISEARCH COPYRIGHT 2000 ISI (R)
TI An evaluation of an anamnestic ELISA for the detection of tuberculous cattle

L11 ANSWER 16 OF 43 SCISEARCH COPYRIGHT 2000 ISI (R)
TI The immunological response of Llamas (Lama glama) following experimental infection with Mycobacterium bovis

L11 ANSWER 17 OF 43 SCISEARCH COPYRIGHT 2000 ISI (R)
TI Sequence heterogeneity of an **mpb70** gene analogue in Mycobacterium kansasii

L11 ANSWER 18 OF 43 SCISEARCH COPYRIGHT 2000 ISI (R)
TI DIFFERENTIAL T-CELL RESPONSES TO MYCOBACTERIA-SECRETED PROTEINS DISTINGUISH VACCINATION WITH BACILLE CALMETTE-GUERIN FROM INFECTION WITH MYCOBACTERIUM-TUBERCULOSIS

L11 ANSWER 19 OF 43 SCISEARCH COPYRIGHT 2000 ISI (R)
TI ETIOLOGY, PATHOGENESIS AND DIAGNOSIS OF MYCOBACTERIUM-BOVIS IN DEER

L11 ANSWER 20 OF 43 SCISEARCH COPYRIGHT 2000 ISI (R)
TI ANTIGENIC CHARACTERIZATION OF MYCOBACTERIUM-BOVIS BCG SOLUBLE-ANTIGENS

L11 ANSWER 21 OF 43 SCISEARCH COPYRIGHT 2000 ISI (R)
TI IDENTIFICATION OF A NOVEL 27-KDA PROTEIN FROM MYCOBACTERIUM-TUBERCULOSIS CULTURE FLUID BY A MONOCLONAL-ANTIBODY SPECIFIC FOR THE MYCOBACTERIUM-TUBERCULOSIS COMPLEX

L11 ANSWER 22 OF 43 SCISEARCH COPYRIGHT 2000 ISI (R)
TI PURIFICATION OF **MPB70** AND PRODUCTION OF SPECIFIC MONOCLONAL-ANTIBODIES

L11 ANSWER 23 OF 43 BIOSIS COPYRIGHT 2000 BIOSIS
TI Cell mediated and humoral immune responses of white-tailed deer experimentally infected with Mycobacterium bovis.

L11 ANSWER 24 OF 43 BIOSIS COPYRIGHT 2000 BIOSIS
TI Investigation of **MPB70** protein presence in BCG moreau substrain

used for production of Polish antituberculosis vaccine.

L11 ANSWER 25 OF 43 BIOSIS COPYRIGHT 2000 BIOSIS
TI The immunological response of llamas (Lama glama) following experimental infection with Mycobacterium bovis.

L11 ANSWER 26 OF 43 BIOSIS COPYRIGHT 2000 BIOSIS
TI Sequence heterogeneity of an **mpb70** gene analogue in Mycobacterium kansasii.

L11 ANSWER 27 OF 43 BIOSIS COPYRIGHT 2000 BIOSIS
TI Differential T cell responses to mycobacteria-secreted proteins distinguish vaccination with bacille Calmette-Guerin from infection with Mycobacterium tuberculosis.

L11 ANSWER 28 OF 43 BIOSIS COPYRIGHT 2000 BIOSIS
TI Identification of a novel 27-kDa protein from Mycobacterium tuberculosis culture fluid by a monoclonal antibody specific for the Mycobacterium tuberculosis complex.

L11 ANSWER 29 OF 43 BIOSIS COPYRIGHT 2000 BIOSIS
TI PURIFICATION OF **MPB70** AND PRODUCTION OF SPECIFIC MONOCLONAL ANTIBODIES.

L11 ANSWER 30 OF 43 BIOSIS COPYRIGHT 2000 BIOSIS
TI CARRIER EFFECT OF CONCANAVALIN A-REACTIVE AND CONCANAVALIN A NON-REACTIVE MATERIAL IN TUBERCULIN PPD.

L11 ANSWER 31 OF 43 BIOSIS COPYRIGHT 2000 BIOSIS
TI PROTEIN G-BASED ELISA FOR ANTI-**MPB70** ANTIBODIES IN BOVINE TUBERCULOSIS.

L11 ANSWER 32 OF 43 BIOSIS COPYRIGHT 2000 BIOSIS
TI **MPB70** A UNIQUE ANTIGENIC PROTEIN ISOLATED FROM THE CULTURE FILTRATE OF BCG SUBSTRAIN TOKYO.

L11 ANSWER 33 OF 43 BIOSIS COPYRIGHT 2000 BIOSIS
TI PROPERTIES OF PROTEINS MPB-64 MPB-70 AND MPB-80 OF MYCOBACTERIUM-BOVIS BCG.

L11 ANSWER 34 OF 43 BIOSIS COPYRIGHT 2000 BIOSIS
TI COMPARATIVE STUDIES WITH VARIOUS SUBSTRAINS OF MYCOBACTERIUM-BOVIS BCG ON THE PRODUCTION OF AN ANTIGENIC PROTEIN MPB-70.

L11 ANSWER 35 OF 43 BIOSIS COPYRIGHT 2000 BIOSIS
TI SPECIFIC **SKIN** REACTIVE PROTEIN FROM CULTURE FILTRATE OF MYCOBACTERIUM-BOVIS BCG.

L11 ANSWER 36 OF 43 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
TI Sequence heterogeneity of an **mpb70** gene analogue in Mycobacterium kansasii.

L11 ANSWER 37 OF 43 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
TI Differential T cell responses to mycobacteria-secreted proteins distinguish vaccination with bacille Calmette-Guerin from infection with Mycobacterium tuberculosis.

L11 ANSWER 38 OF 43 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
TI Identification of a novel 27-kDa protein from Mycobacterium tuberculosis culture fluid by a monoclonal antibody specific for the Mycobacterium tuberculosis complex.

L11 ANSWER 39 OF 43 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
TI Purification of **MPB70** and production of specific monoclonal antibodies.

L11 ANSWER 40 OF 43 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
TI Protein G-based enzyme-linked immunosorbent assay for anti-**MPB70** antibodies in bovine tuberculosis.

L11 ANSWER 41 OF 43 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
TI Properties of proteins MPB64, **MPB70**, and **MPB80** of Mycobacterium bovis BCG.

L11 ANSWER 42 OF 43 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
TI Comparative studies with various substrains of Mycobacterium bovis BCG on the production of antigenic protein, **MPB70**.

L11 ANSWER 43 OF 43 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
TI Specific **skin**-reactive protein from culture filtrate of Mycobacterium bovis BCG.

=> d l11 ab 12,18,30,35

L11 ANSWER 12 OF 43 MEDLINE
AB A highly purified protein, named **MPB70**, was isolated from the culture filtrate of Mycobacterium bovis BCG. This protein accounted for more than 10% of the proteins secreted into the culture medium. **MPB70** was purified by precipitation with ammonium sulfate, followed by treatment with diethylaminoethyl ion exchanger, with or without 3 M urea, and by gel filtration. The final **MPB70** preparation was homogenous as judged by several analyses. The molecular weight was estimated to be 18,000 by electrophoresis or molecular sieve and 15,100 by sedimentation equilibrium. The preparation did not contain sugars. The amino acid composition did not include cysteine or tryptophan.
In **skin** reaction, **MPB70** was a strictly BCG-specific antigen and, among the guinea pigs sensitized with the heat-killed cells of the various species of mycobacteria--Mycobacterium tuberculosis strains H37Rv and Aoyama B, Mycobacterium kansasii, Mycobacterium intracellulare, Mycobacterium phlei, and BCG, it elicited a delayed cutaneous reaction only in the guinea pigs sensitized with BCG. The potency of **MPB70** in the **skin** reaction was about one-twentieth of the standard purified protein derivative.

L11 ANSWER 18 OF 43 SCISEARCH COPYRIGHT 2000 ISI (R)
AB The immune responses of healthy recipients of Mycobacterium bovis bacille Calmette-Guerin (BCG) vaccine, tuberculosis (TB) patients, and contacts of TB patients were examined to three major secretory proteins Mycobacterium tuberculosis, **MPB59**, MPB64, and **MPB70**. **MPB59** evoked a T cell response in 78% of BCG vaccinees, 62% of TB patients, and 60% of contacts. MPB64 and **MPB70** were recognized by <15% of BCG vaccinees, half of TB patients, and three-quarters of contacts. TB and leprosy patients had antibody responses to **MPB59** , but few had antibodies to MPB64 or **MPB70**. Hybridization of mycobacterial DNA with specific gene probes demonstrated the absence of a gene for MPB64 in the vaccine strain of BCG, but the **MPB70** gene was found in all virulent and vaccine BCG strains tested. Since MPB64 and **MPB70** can induce delayed-type hypersensitivity reactions in infected animals, either of these proteins may have potential as **skin** test reagents for detecting infection with M. tuberculosis.

L11 ANSWER 30 OF 43 BIOSIS COPYRIGHT 2000 BIOSIS
AB Tuberculin purified protein derivative (PPD) is a very potent T-cell reactive material in tuberculin-positive individuals, but the components responsible for this reactivity have not been adequately defined. Three purified mycobacterial proteins (**MPB70**, the BCG 65-kDa protein, and BCG antigen 85B) with different degrees of temperature sensitivity were iodine-labelled and added to BCG culture fluid, and the mixtures were

autoclaved at 120.degree.C for 30 min to simulate the initial heating procedure used to prepare PPD. SDS-PAGE followed by protein staining and autoradiography showed that the banded pattern of unheated culture fluid was completely lost after heating, and only the labelled **MPB70** preparation retained most of the radioactivity in a fraction with soluble protein of the same size. Most mycobacterial proteins are extensively denatured by these procedures, which explains the previous extensive difficulties in isolating defined constitutents from PPD to characterize their behaviour in B- and T-cell reactions. In assays for the carrier effect of NIP-PPD for induction of anti-NIP production in BCG-vaccinated mice, the active fractions were heterogeneous in lectin reactivity as well as in SDS-PAGE. It appears most likely that a number of Mycobacterium tuberculosis proteins gave rise to core peptides which resist proteolysis and heat denaturation to possess powerful T-cell-activating ability.

L11 ANSWER 35 OF 43 BIOSIS COPYRIGHT 2000 BIOSIS
AB A highly purified protein, named **MPB70**, was isolated from the culture filtrate of M. bovis BCG. This protein accounted for > 10% of the proteins secreted into the culture medium. **MPB70** was purified by precipitation with ammonium sulfate, followed by treatment with DEAE ion exchanger, with or without 3 M urea, and by gel filtration. The final **MPB70** preparation was homogenous as judged by several analyses. The MW was estimated to be 18,000 by electrophoresis or molecular sieve and 15,100 by sedimentation equilibrium. The preparation did not contain sugars. The amino acid composition did not include cysteine or tryptophan.
In **skin** reaction, **MPB70** was a strictly BCG-specific antigen and, among the guinea pigs sensitized with heat-killed cells of various species of mycobacteria (M. tuberculosis strains H37Rv and Aoyama B, M. kansasii, M. intracellulare, M. phlei and BCG), it elicited a delayed cutaneous reaction only in the guinea pigs sensitized with BCG. The potency of **MPB70** in the **skin** reaction was about 1/20 of the standard purified protein derivative.

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(FILE 'HOME' ENTERED AT 15:52:17 ON 14 JUN 2000)

FILE 'MEDLINE, SCISEARCH, BIOSIS, EMBASE' ENTERED AT 15:52:51 ON 14 JUN 2000

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L1            0 S MPB64 AND (TWEEN 80)
L2            0 S (TRANSDERMAL DEVICE?) AND TUBERCULOSIS AND ANTIGEN?
L3            0 S (TRANSDERMAL DEVICE?) AND TUBERCULOSIS
L4         3410 S (TRANSDERM? OR TRANSPORT?) AND TUBERCULOSIS
L5            0 S L4 AND (TOPIC? APPLICATION)
L6            9 S L4 AND (TOPIC?)
L7            0 S L4 AND MPB64
L8          255 S MPB44 OR MPB45 OR MPB51 OR MPB59 OR MPB70 OR MPB80 OR MPB84
L9            4 S L4 AND L8
L10           0 S L8 AND TRANSDERM?
L11          43 S L8 AND SKIN
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